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Acute and Subacute Toxicity of 7.5% Hypertonic Saline–6% Dextran-70 (HSD) in Dogs 2. Biochemical and Behavioral Responses*

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7.5% Hypertonic saline–6% Dextran-70 (HSD) is currently being evaluated in our laboratory as a resuscitation solution for the treatment of hypovolemia at a dose of 4 ml kg^{-1} body weight. A few reports of dextran toxicity, particularly of the kidney, have been cited in the literature, so the present study evaluated the acute and subacute toxicity of HSD administered *i.v.* to beagle dogs. In the acute toxicity studies animals were infused with a single dose of HSD, or its components of hypertonic saline (HS) or Dextran-70 (D-70), at the maximum tolerated dose (MTD: 20 ml kg^{-1}). Controls received Ringers lactate (RL). In the HSD-infused dogs, transient but significant increases in serum alanine (ala) aminotransferase (AT), aspartate (asp) AT and alkaline phosphatase (AP) were observed for the first 72 h. In most cases this increase was also observed in the HS group. In the subacute studies, dogs were infused daily with the MTD of the above test solutions. Serum ala AT activity was 2–3-fold higher in the HSD than the RL group for the first 3 days. Again, a similar effect was observed in the HS group. Slight, transient increases in asp AT and AP activity were also observed in the HSD group. Higher lactate dehydrogenase (LDH) activity was only observed at Day 14 in dogs infused with the MTD of HSD or HS. In both studies, no adverse effects on blood urea nitrogen (BUN) or serum creatinine were observed and other transient changes in serum parameters were attributable to hemodilution induced by HSD. No gross or microscopic lesions were observed in any major organ. Considering that the proposed therapeutic dose of HSD is only 4 ml kg^{-1}, it appears that its use should be associated with minimal or no adverse effects.

INTRODUCTION

Over the past decade studies have shown that hypertonic crystalloid solutions, alone or in combination with dextran, can be an effective small-volume resuscitation solution for the treatment of hypovolemic states.[1–3] Consequently, the introduction of HSD (7.5% NaCl–6% Dextran-70) has generated a number of experimental studies investigating its safety and efficacy,[4–6] and a new drug application (NDA) for HSD is currently under review by the US Food and Drug Administration. Under the provisions of the NDA a single dose of 250 ml of HSD (ca. 4 ml kg^{-1}) would be administered for the initial treatment of hemorrhagic hypovolemia. In the military setting for the treatment of battlefield casualties, possible multiple doses of HSD may be considered.

Before HSD can be incorporated into a rational therapeutic regime, concerns over the potential toxicity of the dextran and hypertonic saline components of HSD must be addressed. In the nearly 50 years that dextrans have been employed as plasma volume expanders, few reports of their general toxicity have appeared in the literature. Associated side-effects of dextran use have been attributed mostly to allergic reactions[7–11] or to disturbances in hemostasis.[7,12–16] In general, these effects have been attributed to doses exceeding 1 l of high-molecular-weight dextrans (>100 000). On the other hand, reports of dextran-induced renal toxicity have been associated with repeated administration of high doses of dextran with an average molecular size distribution of 40 000.[17,18] Studies in rabbits have shown that repeated administration of large doses of Dextran-70 resulted in dextran accumulation in white blood cells, liver and spleen, yet no evidence for dextran-induced tissue damage has been observed.[19,20] Acute toxicity studies with dextrans at repeated doses of 10–80 ml kg^{-1} in mice, dogs and rabbits have shown circulatory overload as the primary adverse effect and overall toxicity of i.v. administered dextran was extremely low (G. Jonsson, personal communication).[21]

Use of hypertonic salt solutions has primarily raised concern over the potential neurological effects induced by hypernatremia. Thus, as part of the new drug application to FDA for HSD, the present studies investigated the acute and subacute toxicity of HSD and its individual components in dogs. Particular attention was focused on evidence for renal or hepatic toxicity, as well as behavioral changes.

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* The opinions and assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. (AR 360-5.)

In conducting the research described in this report, the investigators adhered to the 'Guide for the Care and Use of Laboratory Animals', as promulgated by the Committee on revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animal Resources, National Research Council, Bethesda, MD, USA.

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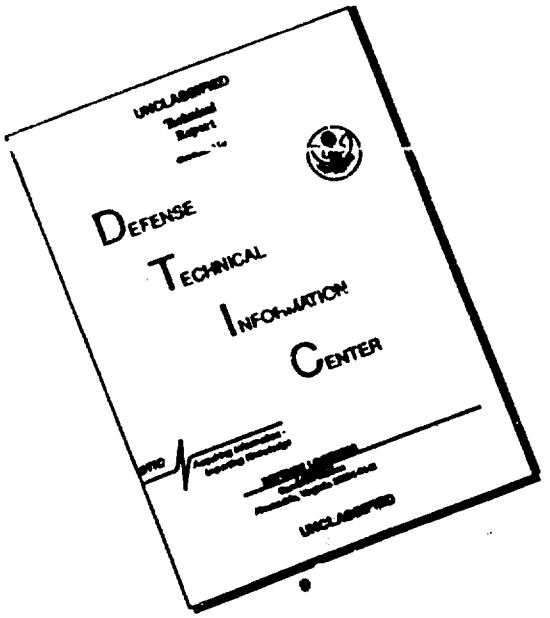

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EXPERIMENTAL

Animals and treatment

Separate acute and subacute toxicity studies were conducted under Good Laboratory Practices (GLP) conditions. In the acute studies, male ($n = 8$) and female ($n = 8$) beagle dogs, weighing initially 7.5–10.9 kg, were obtained from Ridglan Farms, Inc. (Mt. Horeb, WI). They were individually housed in stainless-steel runs and fed Purina Canine Diet 5007 and purified water *ad libitum*. For the daily dosing subacute toxicity studies, 60 beagle dogs (30 male, 30 female) weighing 8.7–14.4 kg were obtained from Hazelton-LRE (Kalamazoo, MI). Details pertaining to animal housing and husbandry have been described previously.[22,23]

In the single-dose acute toxicity studies, dogs were infused i.v. via the cephalic or saphenous vein with the maximum tolerated dose (MTD) of 20 ml kg^{-1} HSD (Lot NC54845) or its individual components, 6% Dextran-70 (D-70; Lot NE54941) or 7.5% NaCl (HS; Lot I318712). Ringer's lactate (RL; Lot NC54847) was used as the control solution. All solutions were obtained from Pharmacia AB (Uppsala, Sweden) and were prepared under strict adherence to Good Manufacturing Practice guidelines. In the studies in which dogs received daily infusions of 12, 16 and 20 ml kg^{-1}, solutions were administered i.v. over a 5-min period.

In dogs infused with a single dose of HSD or D-70, blood samples were withdrawn prior to and 0.25, 1, 2, 3, 7 and 14 days following infusion. Blood samples in the multiple dosing studies were withdrawn prior to dosing (baseline) and on days 1, 2, 3, 7 and 14 prior to the infusions. Serum was separated from blood cells by centrifugation and stored at −20°C until analyzed.

Observations and serum chemistry

During the 14-day observation period, clinical observations were accomplished daily before dosing, 1 h after dosing and in the afternoon. All observations were performed by the same technician in order to minimize interpersonnel variation in the detection of behavioral disturbances. All observations complied with Letterman Army Institute of Research Standard Operating Procedures defining clinical observations and their documentation in toxicology studies.

Serum enzyme determinations, blood urea nitrogen (BUN), creatinine and assays for sodium, potassium and chloride were performed by the Analytical Chemistry Branch, Letterman Army Institute of Research. Commercial assays for alanine and aspartate aminotransferases (ala AT; asp AT), lactate dehydrogenase (LDH), alkaline phosphatase (AP), creatine kinase (CK) and gamma-glutamyl transpeptidase (GGTP), BUN, creatinine and chloride were adapted for use on a Cobas Fara II centrifugal fast analyzer (Roche Analytical Instruments, Belleville, NJ). Serum sodium and potassium concentrations were determined with a flame photometer (Instrumentation Laboratory, Lexington, MA).

Table 1. Clinical observations following a single bolus infusion (20 ml kg^{-1}) in dogs[a]

Observation	Solution infused			
	RL	HSD	HS	D-70
Behavioral	0/4	4/4	4/4	2/4
Gastrointestinal	0/4	4/4	3/4	1/4

[a] Data represent number of animals observed/number of animals per group.

Statistical analysis

Data were analyzed by repeated-measure ANOVA, with treatment and time as the independent variables. Differences among groups were further analyzed by Newman–Keuls multiple range test.[24] $P < 0.05$ was considered to be statistically significant.

RESULTS

Acute studies

Clinical observations. In the acute dog studies, clinical signs observed were grouped into behavioral and gastrointestinal categories (Table 1). With the exception of two cases of diarrhea and one case of tremors, all clinical signs were observed on Day 0 immediately following dosing and resolved to normal within 24 h. Dogs receiving HSD or HS exhibited the greatest incidence of signs. No clinical signs were observed in animals receiving RL.

Behavioral signs were the most frequently observed category. Inactivity (9 of 16 dogs) was observed in all animals receiving HSD and HS, but only in one animal receiving D-70. By 4 h after dosing, one HSD- and one HS-treated animal remained inactive. At the next observation period, 24 h after dosing, all had returned to normal activity levels. Disorientation was observed in dogs receiving HSD (four animals) and D-70 (two animals), but resolved by 2 h in both groups. Tremors (6 of 16) were observed in the HS (four animals) and HSD (two animals) groups. One animal receiving HS exhibited tremors on Day 1, but the animal returned to normal by Day 2. Ataxia was observed on Day 0 in one animal receiving HS.

Gastrointestinal signs observed included vomiting (7 of 16 animals), excessive thirst (2 of 16), increased salivation (6 of 15) and diarrhea (2 of 16) (Table 1), which were seen primarily in animals receiving HSD and HS. The diarrhea observed was not related to the time of dosing.

Serum chemistry. In dogs infused with a single acute dose of HSD or HS at the MTD, serum Na and K concentrations at 0.25 days following infusion were significantly higher and lower, respectively, in comparison to the other groups (Table 2). At the other times, Na and K group concentrations were similar among the groups throughout the experimental period.

In dogs infused with a single dose of HSD at the

Table 2. Serum electrolytes in dogs infused with 20 ml kg^{-1} of the test solutions

		Day 0	0.25	1	3	7	14
Na (mEq l^{-1})	RL	153 ± 1	152 ± 2	152 ± 2	155 ± 2	150 ± 5	153 ± 2
	HSD	152 ± 2	157 ± 1*	152 ± 1	152 ± 3	151 ± 1	145 ± 2
	D-70	152 ± 2	151 ± 2	152 ± 2	151 ± 1	149 ± 1	150 ± 1
	HS	152 ± 2	156 ± 1*	151 ± 2	151 ± 1	138 ± 5	149 ± 3
K (mEq l^{-1})	RL	5.3 ± 0.1	5.1 ± 0.2	4.8 ± 0.2	5.0 ± 0.2	4.8 ± 0.2	5.0 ± 0.2
	HSD	5.2 ± 0.1	4.1 ± 0.1*	4.7 ± 0.1	5.0 ± 0.2	5.2 ± 0.2	4.8 ± 0.2
	D-70	5.1 ± 0.2	5.1 ± 0.1	4.8 ± 0.2	4.8 ± 0.2	4.8 ± 0.2	4.8 ± 0.2
	HS	5.1 ± 0.2	4.4 ± 0.1*	4.6 ± 0.1	4.8 ± 0.1	4.3 ± 0.2*	4.8 ± 0.2
Cl (mEq l^{-1})	RL	111 ± 1	115 ± 1	111 ± 1	116 ± 1	114 ± 2	113 ± 2
	HSD	110 ± 1	116 ± 2	112 ± 1	113 ± 2	112 ± 1	109 ± 4
	D-70	113 ± 1	114 ± 1	113 ± 1	113 ± 1	114 ± 1	113 ± 1
	HS	112 ± 1	116 ± 1	111 ± 1	114 ± 1	107 ± 3	112 ± 2

* Data expressed as means ± SE for four dogs per group.
* $P < 0.05$ from Day 0 value and corresponding RL and D-70 groups.

MTD, a transient rise in serum ala AT, asp AT and AP was observed for the first 2–3 days following infusion (Figs 1 and 2). No differences in LDH activity were observed following HSD infusion or its individual components (Fig. 2). Alanine AT was also elevated in serum from HS-infused dogs for the first 2 days following infusion, while AP activity was elevated in serum for up to 3 days following D-70 infusion (Figs 1 and 2). Creatine kinase and GGTP activities in serum were not significantly affected by infusion of HSD or its components (data not shown).

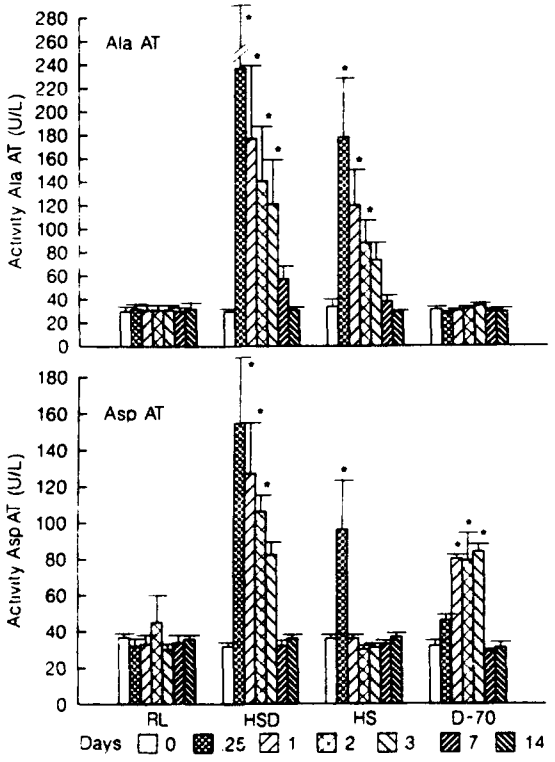

Figure 1. Alanine and aspartate aminotransferase activity in serum from dogs infused with a single dose of 20 ml kg^{-1} (MTD) of HSD or its components. Data expressed as means ± SE for four dogs per group. *$P < 0.05$ from baseline and RL controls.

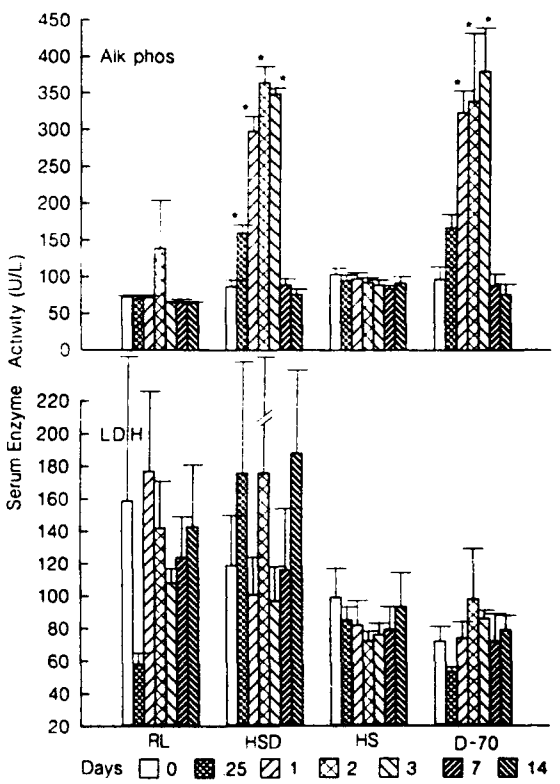

Figure 2. Alkaline phosphatase and lactate dehydrogenase activity in serum from dogs infused with the MTD of HSD or its components. Data expressed as means ± SE for four dogs per group. *$P < 0.05$ from baseline and RL controls.

Table 3. Effect of a single MTD of HSD or its components on blood urea nitrogen and serum creatinine concentrations[a]

		Day						
		0	0.25	1	2	3	7	14
BUN	RL	19.3 ± 2.4	20.0 ± 1.6	15.6 ± 1.6	16.8 ± 0.9	18.7 ± 2.4	18.4 ± 2.2	14.4 ± 1.8
(mg dl^{-1})	HSD	16.3 ± 1.2	12.4 ± 2.3*	12.9 ± 1.6	16.4 ± 2.5	14.8 ± 0.7	17.4 ± 3.3	13.8 ± 0.9
	D-70	17.2 ± 2.6	20.0 ± 1.6	15.3 ± 1.3	20.3 ± 2.6	17.2 ± 1.1	16.8 ± 2.7	15.6 ± 1.4
	HS	16.6 ± 1.6	15.0 ± 0.7	14.1 ± 1.2	17.3 ± 1.9	16.5 ± 1.1	17.8 ± 1.1	13.8 ± 0.8
Creatinine	RL	0.73 ± 0.10	0.68 ± 0.06	0.73 ± 0.02	0.70 ± 0.07	0.75 ± 0.06	0.75 ± 0.06	0.80 ± 0.07
(mg dl^{-1})	HSD	0.68 ± 0.05	0.55 ± 0.03*	0.75 ± 0.06	0.70 ± 0.08	0.68 ± 0.08	0.68 ± 0.05	0.73 ± 0.06
	D-70	0.70 ± 0.04	0.65 ± 0.03	0.80 ± 0.04	0.70 ± 0.04	0.73 ± 0.05	0.73 ± 0.02	0.80 ± 0.04
	HS	0.65 ± 0.03	0.50 ± 0.0*	0.75 ± 0.03	0.75 ± 0.03	0.73 ± 0.02	0.60 ± 0.04	0.78 ± 0.02*

[a] Data expressed as means ± SE for four animals per group.
* $P < 0.05$ from Day 0 baseline values.

In these animals, BUN was significantly lower than baseline values 0.25 day following HSD infusion, while serum creatinine concentrations were lower at this time in both the HSD and HS groups (Table 3). Blood urea nitrogen and creatinine were not significantly different among the groups at the other time points (Table 3).

Subacute studies

Clinical observations. In the subacute studies, the clinical signs observed were also grouped into behavioral and gastrointestinal categories (Table 4); 'Other' and 'General' categories were also included, which primarily represented respiratory disturbances. With the exception of soft stool, which exhibited an equivalent incidence among all groups, all major clinical signs were observed with greatest incidence in dogs receiving HSD or HS. The incidence of each individual sign was approximately the same among the HSD- and HS-treated groups. The incidence of major signs in D-70-treated groups was intermediate between the HSD- or HS-treated groups and those treated with RL, and were not dose related. No sex-related differences were apparent in any of the clinical observations.

Table 4. Clinical observations following daily infusions in dogs[a]

	Dose	Solution infused			
Observation	(ml kg^{-1})	RL[b]	HSD	HS	D-70
Behavioral	12		6/6	6/6	4/6
	16		6/6	6/6	3/6
	20	2/6	5/6	6/6	4/6
Gastrointestinal	12		6/6	6/6	1/6
	16		6/6	6/6	2/6
	20	4/6	6/6	6/6	6/6
General	12		5/6	5/6	2/6
	16		6/6	6/6	2/6
	20	1/6	6/6	6/6	1/6
Other	12		1/6	2/6	2/6
Panting	16		0/6	1/6	2/6
Nasal	20	0/6	1/6	1/6	2/6

[a] Data presented as number of observations:number of animals per group.
[b] Ringers lactate was only administered at 20 ml kg^{-1}.

Behavioral disturbances were also the most frequent clinical observations in the subacute studies (Table 4). Behavioral signs observed included disorientation (48 of 60 animals), inactivity (47 of 60), tremors (40 of 60), hyperactivity (6 of 60), pacing (5 of 60), circling (3 of 60) and staggering (2 of 60). Disorientation, inactivity and tremors were observed with greatest incidence in HSD- and HS-treated animals. A moderate incidence of these three signs was observed in D-70-treated animals, while the signs appeared only sporadically among those receiving RL. The incidence and severity of behavioral signs were greatest one hour after dosing each day and generally resolved within 24 h after dosing. The signs reappeared after the next day's dosing, and repeated the cycle of resolution over the following 24 h. Hyperactivity, pacing, circling and staggering occurred sporadically throughout the study period and were randomly distributed among the groups.

General signs observed included excessive thirst (32 of 60), hunched posture (31 of 60), increased salivation (29 of 60), decreased appetite (15 of 60), excessive bleeding from the injection site (2 of 60), swelling or edema of the injected leg (2 of 60) and bloody urine (1 of 60) (Table 4). Excessive thirst, hunched posture and increased salivation were observed primarily in animals receiving HSD or HS, but were also seen in D-70- and RL-treated animals. Increased salivation was the only sign that appeared to be dose related, and occurred primarily in the middle- and high-dose HSD or HS groups. Many animals developed a conditioned response and would begin to salivate when removed from their run in preparation for dosing. The salivation generally subsided by the afternoon observation period. Decreased appetite was distributed equally among the groups. Swelling of the injected leg was observed only in HSD- or HS-treated animals and resolved within 48 h in each case.

Gastrointestinal signs included vomiting (40 of 60), soft stool (37 of 60) and diarrhea (2 of 60) (Table 4). Vomiting was observed in all HSD- (18 of 18) and HS-treated (18 of 18) animals and usually occurred within 1 h after dosing. Vomiting occurred with a lower incidence in D-70-treated animals (4 of 18) and

Table 5. Serum electrolytes in dogs infused daily with the MTD of each test solution*

		Day 0	1	3	7	14
Na (mEq l^{-1})	RL	115 ± 2	153 ± 1	152 ± 1	115 ± 1	152 ± 1
	HSD	153 ± 1	156 ± 2	151 ± 1	152 ± 1	151 ± 1
	D-70	154 ± 1	155 ± 2	148 ± 4	152 ± 1	150 ± 1
	HS	153 ± 1	153 ± 3	153 ± 1	154 ± 2	153 ± 1
K (mEq l^{-1})	RL	4.8 ± 0.2	4.8 ± 0.2	4.8 ± 0.1	4.8 ± 0.1	4.9 ± 0.1
	HSD	4.9 ± 0.2	4.7 ± 0.1	4.8 ± 0.1	4.4 ± 0.1	4.6 ± 0.1
	D-70	4.9 ± 0.1	4.7 ± 0.1	4.6 ± 0.2	4.4 ± 0.1	4.6 ± 0.1
	HS	4.8 ± 0.2	4.6 ± 0.1	4.8 ± 0.1	4.9 ± 0.2	5.0 ± 0.1
Cl (mEq l^{-1})	RL	116 ± 1	116 ± 1	115 ± 1	117 ± 1	116 ± 1
	HSD	114 ± 1	120 ± 2	116 ± 1	117 ± 1	118 ± 1
	D-70	116 ± 1	118 ± 1	117 ± 1	119 ± 1	118 ± 1
	HS	114 ± 1	119 ± 1	114 ± 1	115 ± 2	113 ± 1

* Data expressed as means ± SE for six dogs per group.

was not observed at all in animals treated with RL. The frequency of vomiting for Week 2 was approximately half of that observed in the first week. Diarrhea was observed only in two HSD-treated animals, and resolved within 48 h in each case.

Respiratory signs included increased respiratory depth (15 of 60), panting (12 of 60), increased respiratory rate (3 of 60) and congestion (3 of 60) (Table 4). Increased respiratory depth was observed with greatest incidence in animals treated with HSD (7 of 18), followed by those treated with HS (5 of 18) and D-70 (3 of 18). Panting was most prevalent in animals receiving D-70 (6 of 18), followed by HS (4 of 18) and HSD (2 of 18). Increased respiratory rate and congestion were sporadically observed among the groups. No respiratory signs were observed in animals receiving RL.

Serum chemistry. Serum Na, K and Cl concentrations were similar in all groups throughout the experimental period (Table 5). Because there were no differences among groups at all the doses infused, data are only presented from animals that received the MTD.

Serum enzyme activity following daily infusion of HSD at the MTD for 14 days responded in a similar way to that in the acute studies, except that the elevation in ala AT, asp AT and AP continued throughout the experimental period (Figs 3 and 4). Since the highest elevations in serum enzyme concentrations were observed in the dogs infused daily at the MTD, only those data are depicted.

In dogs infused daily at the MTD of HSD or its individual components, HS or D-70, no significant differences in serum creatinine or BUN were observed among the groups throughout the 14-day experimental period (Table 6).

DISCUSSION

In the present study, 6 h following infusion of a single bolus of the MTD (20 ml kg^{-1}) of HSD to euvolemic dogs, serum Na and K concentrations were significantly

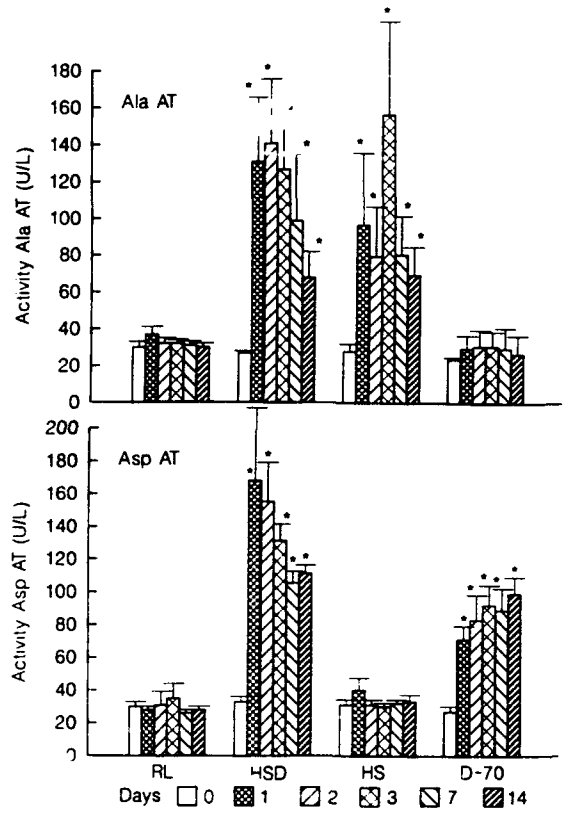

Figure 3. Alanine and aspartate aminotransferase activity in serum from dogs infused daily for 14 days with the MTD of HSD or its components. Data expressed as means ± SE for six dogs per group. *$P < 0.05$ from baseline and RL controls.

higher and lower, respectively, than pre-infusion levels, although peak concentrations were not determined. At 24 h, Na and K concentrations had returned to normal. This is in agreement with previous studies showing a peak rise in serum Na within minutes after an infusion of HSD or hypertonic saline and then a slow return of serum Na towards normal levels.[1,3,4] This return to baseline occurs more rapidly in animals allowed free access to water following HSD infusion, and under these conditions Na concentrations are normal by 24 h after infusion.[4] Since animals in the present study were allowed free access to water, it is not surprising that serum Na concentrations are normal 24 h after HSD infusion.

In addition, the decrease in serum K observed in dogs 6 h following a single MTD of HSD was transient and was not clinically significant. However, since the hematocrit and serum protein concentrations were not affected 6 h after HSD infusion,[25] this effect on serum K cannot be readily explained by simple hemodilution and may be related to increased renal excretion of Na and K following HSD infusion. However, urine was not collected in this study to confirm this hypothesis.

From the discussion above it is not surprising that serum Na and K concentrations did not appear to be affected by daily dosing, since blood samplings were made about 24 h after each subsequent dose. In addition, serum creatinine and BUN concentrations

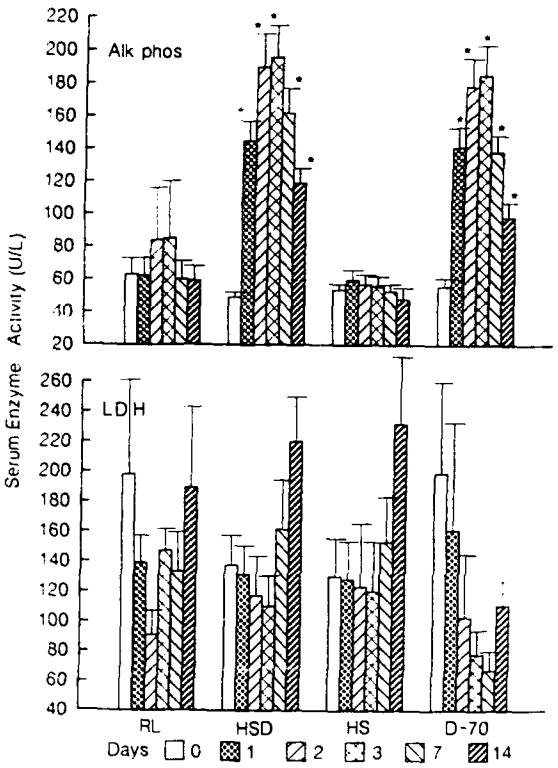

Figure 4. Alkaline phosphatase and lactate dehydrogenase activity in serum from dogs infused daily for 14 days with the MTD of HSD or its components. Data expressed as means ± SE for six dogs per group. *$P < 0.05$ from baseline and RL controls.

infusion protocols in rabbits.[26,27] In rabbits, however, HS infusion at the MTD induced the greatest behavioral disturbances and was lethal within 12 min of infusion in 6 of 20 rabbits.[26] Although symptoms resembling seizures were not observed in these studies, induction of seizures is a major clinical concern in response to hypernatremia. In the present study, a cause–effect relationship between hypernatremia and behavioral disturbances can only be inferred by the higher incidence of abnormalities in the HSD and HS groups, but blood samples were not collected early enough to determine the peak serum Na concentrations. In recent preliminary studies in sheep infused with 4 ml kg^{-1} of HS solutions ranging from 3% to 25%, plasma Na concentrations exhibited a dose-dependent increase. In the 25% HS group, plasma Na concentrations exceeded 200 mEq l^{-1} within 2–3 min following infusion and then quickly fell to more clinically acceptable levels after 1 h.[28] Since the total Na load in the present studies is greater than in this sheep study, it is quite possible that the transient behavioral disturbances observed are related to hypernatremia. However, this would require verification. Nevertheless, the other symptoms are consistent with volume and fluid overload previously observed in toxicity studies with earlier clinical dextran preparations.[21,26]

In the single-dose studies at the MTD, serum enzyme levels, as a clinical profile for liver damage, showed only transient changes. Similar results were observed in the rabbit studies.[26] A number of earlier studies have shown that infused dextran can accumulate in the liver[19,20,29–32] and we have recently shown that, following infusion of 4 ml kg^{-1} HSD, < 10% remains in the liver after 4 days.[33] Despite this storage in liver and other tissues, dextran does not seem to be

Table 6. Effects of daily infusions of the MTD of HSD and its components on blood urea nitrogen and serum creatinine[a]

		Day				
		0	1	3	7	14
BUN (mg dl^{-1})	RL	17.5 ± 2.5	15.4 ± 0.7	17.8 ± 1.5	19.0 ± 1.4	17.6 ± 1.0
	HSD	16.1 ± 0.4	13.2 ± 0.7	16.8 ± 1.1	16.8 ± 0.8	16.4 ± 0.8
	D-70	16.0 ± 0.8	16.4 ± 1.4	16.8 ± 1.4	16.9 ± 2.4	16.4 ± 0.9
	HS	17.9 ± 2.2	15.6 ± 1.7	17.9 ± 1.2	17.5 ± 1.5	19.3 ± 1.0
Creatinine (mg dl^{-1})	RL	0.68 ± 0.02	0.60 ± 0.09	0.70 ± 0.04	0.72 ± 0.04	0.82 ± 0.03
	HSD	0.75 ± 0.03	0.72 ± 0.05	0.68 ± 0.03	0.72 ± 0.05	0.70 ± 0.06
	D-70	0.72 ± 0.02	0.68 ± 0.04	0.67 ± 0.03	0.68 ± 0.03	0.68 ± 0.05
	HS	0.77 ± 0.02	0.75 ± 0.02	0.72 ± 0.02	0.73 ± 0.05	0.83 ± 0.06

[a] Data expressed as means ± SE for six animals per group.

support the idea that even daily dosing of HSD at the MTD does not compromise renal function.

In the present study, infusion of a single dose of HSD or its constituents at the MTD (five times the proposed therapeutic dose)[5,6] induced some degree of behavioral abnormalities, although these were transient in nature. These effects were more pronounced in the HSD and HS groups than the other groups, but these effects were more uniformly distributed among all the groups in the daily dosing subacute toxicity studies. A behavioral profile similar to HSD and its constituents in the present study was observed in our laboratory under the same

associated with any adverse effects on liver function[34] and it is completely catabolized in time.[29] It should be noted that no significant change in serum aminotransferases or LDH were observed following infusion of the estimated therapeutic dose of 4 ml kg^{-1} HSD in swine (Dubick *et al.*, unpublished observations).

Daily infusion of HSD or its components induced a more sustained increase in serum enzyme concentrations, although the levels did begin to decrease after the third day. A similar profile was also observed in our earlier study in rabbits.[26] Although the daily

dosing studies were not extended to investigate the reversibility of the enzyme response once the infusions stopped, both gross and microscopic examination of liver and other major organs failed to detect signs of tissue damage (data not shown). In addition, it is important to note that in the multiple-infusion studies, serum enzyme levels never exceeded the values observed in the acute toxicity studies. These data would imply that the effects of multiple infusions of HSD or its components are not additive.

In conclusion, since the proposed therapeutic dose of HSD is a single infusion of 4 ml kg^{-1}, the present data suggest that its use should be associated with minimal toxicity.

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REFERENCES

1. I. T. Velasco, V. Pontierri and M. Rocha e Silva, Hyperosmotic NaCl and severe hemorrhagic shock. *Am. J. Physiol.* **239**, H664–H673 (1980).
2. P. A. Maningas, L. R. DeGuzman, F. J. Tillman, C. S. Hinson, K. J. Priengnitz, K. A. Volk and R. F. Bellamy, Small-volume infusion of 7.5% NaCl in 6% Dextran 70 for the treatment of severe hemorrhagic shock in swine. *Ann. Emerg. Med.* **15**, 1131–1137 (1986).
3. R. Hands, J. W. Holcroft, P. R. Perron and G. C. Kramer, Comparison of peripheral and central infusion of 7.5% NaCl/6% Dextran-70. *Surgery* **103**, 684–689 (1988).
4. G. C. Kramer, T. P. English, R. A. Gunther and J. W. Holcroft, Physiological mechanisms of fluid resuscitation with hyperosmotic/hyperoncotic solutions. In *Perspectives in Shock Research: Metabolism, Immunology, Mediators and Models*, ed. by J. C. Passmore, S. M. Richard, D. G. Reynolds and D. L. Traber, pp. 311–320. Alan R. Liss, New York (1989).
5. M. A. Dubick, J. J. Summary, B. A. Ryan and C. E. Wade, Dextran concentrations in plasma and urine following administration of 6% Dextran-70/7.5% NaCl to hemorrhaged and euvolemic conscious swine. *Circ Shock* **29**, 301–310 (1989).
6. C. E. Wade, J. P. Hannon, C. A. Bossone, M. M. Hunt, J. A. Loveday, R. Coppes and V. L. Gildengorin, Resuscitation of conscious pigs following hemorrhage: comparative efficacy of small volume resuscitation. *Circ. Shock* **29**, 193–204 (1989).
7. J. M. Mishler IV, Synthetic plasma volume expanders—their pharmacology, safety and clinical efficacy. *Clin. Hematol.* **13**, 75–92 (1984).
8. E. A. Kabat and A. E. Bezer, The effect of variation in molecular weight on the antigenicity of dextran in man. *Arch. Biochem. Biophys.* **78**, 306–318 (1958).
9. G. Bailey, R. L. Strub, R. C. Klein and J. Salvaggio, Dextran-induced anaphylaxis. *J. Am. Med. Assoc.* **200**, 889–891 (1967).
10. H. Hedin, W. Richter and J. Ring, Dextran-induced danaphylactoid reactions in man. Role of dextran reactive antibodies. *Int. Arch. Allergy Appl. Immunol.* **52**, 145–159 (1976).
11. R. Brisman, L. C. Parks and J. Alex Haller, Jr., Anaphylactoid reactions associated with the clinical use of Dextran 70. *J. Am. Med. Assoc.* **204**, 824–825 (1968).
12. B. Alexander, K. Odake, D. Lawlor and M. Swanger, Coagulation, hemostasis, and plasma expanders: a quarter century enigma. *Fed. Proc.* **34**, 1429–1440 (1975).
13. D. Bergqvist, Dextran and hemostasis. *Acta Chir. Scand.* **148**, 633–640 (1982).
14. B. Alexander, Effects of plasma expanders on coagulation and hemostasis: dextran, hydroxyethyl starch and other macromolecules revisited, In *Blood Substitutes and Plasma Expanders*, ed. by G. A. Jamieson and T. J. Greenwalt, Vol. 19, pp. 293–346. Liss, NY (1978).
15. U. Jacobaeus, The effect of dextran on the coagulation of blood. *Acta Med. Scand.* **151**, 505–507 (1955).
16. S. E. Bergentz, O. Eiken and I. M. Nilsson, The effect of dextran of various molecular weight on coagulation in dogs. *Thromb. Diath. Hemorrh.* **6**, 15–24 (1961).
17. J. L. Data and A. S. Nies, Dextran 40, *Ann. Intern. Med.* **81**, 500–504 (1974).
18. B. R. C. Kurnik, F. Singer and W. C. Groh, Case report: dextran-induced acute anuric renal failure, *Am. J. Med. Sci.* **302**, 28–30 (1991).
19. B. H. Persson, Histochemical studies on the fate of parenterally administered dextran in rabbits. 2. On the accumulation of dextran within the kidney, liver, leucocytes and reticuloendothelial system. *Acta Soc. Med. Ups.* **57**, 421–437 (1952).
20. U. Friberg, W. Graf and B. Aberg, Effects of prolonged dextran administration to rabbits. *Acta Pharmacol. Toxicol.* **9**, 220–234 (1953).
21. G. Jonsson, personal communication to C.E.W. (1986).
22. D. F. Frost, G. M. Zaucha, S. T. Omaye, C. B. Clifford and D.W. Korte, Jr., Acute intravenous toxicity study of hypertonic saline/Dextran-70 and its constituents in beagle dogs. Letterman Army Institute of Research Institute Report 392, Presidio of San Francisco, CA (1989).
23. G. M. Zaucha, D. F. Frost, S. T. Omaye, C. B. Clifford and D. W. Korte, Jr., Fourteen day subacute intravenous toxicity study of hypertonic/saline Dextran-70 and in constituents in beagle dogs. Letterman Army Institute of Research Institute Report 404, Presidio of San Francisco, CA (1989).
24. J. Neter and W. Wasserman, *Applied Linear Statistical Models*. Richard D. Irwin, Homewood, IL (1974).
25. J. J. Summary, M. A. Dubick, G. M. Zaucha, A. F. Kilani, D. W. Korte, Jr. and C. E. Wade, Acute and subacute toxicity of 7.5% hypertonic saline 6% Dextran-70 in dogs. 1. Serum immunoglobulin and complement responses. *J. Appl. Toxicol.* **12**, 261–266 (1992).
26. G. M. Zaucha, D. F. Frost, L. McKinney and D. W. Korte, Jr., Acute intravenous toxicity study of hypertonic saline/Dextran-70 and its constituents in New Zealand white rabbits, Letterman Army Institute of Research Report 364, Presidio of San Francisco, CA (1989).
27. G. M. Zaucha, D. F. Frost, S. T. Omaye, L. McKinney, M. J. Pearce and D. W. Korte, Jr., Fourteen day subacute intravenous toxicity study of hypertonic saline/Dextran-70 and its constituents in New Zealand white rabbits. Letterman Army Institute of Research Report 405, Presidio of San Francisco, CA (1989).
28. M. A. Dubick, J. J. Summary, J. M. Davis, J. Y. Greene, C. E. Wade and G. C. Kramer, Dose response comparison between hyperosmotic saline (HS) and hyperoncotic Dextran-70 (HD) as plasma volume expanders (Abstract no. 91). *Circ. Shock* **34**, 37 (1991).
29. U. F. Gruber, *Blood Replacement*. Springer-Verlag, Berlin (1969).
30. B. Swedin and B. Aberg, On dextran in spleen, liver and muscle after intravenous injection into rabbits. *Scand. J. Clin. Lab. Invest.* **4**, 68–70 (1952).
31. J. Linder, Morphology. *Bibl. Haematol.* **37**, 279–298 (1971).
32. M. Eckstein and J. Lindner, The histochemistry of the plasma substitution especially with dextran. *Ann. Histochim.* **7**, 163–176 (1962).
33. M. A. Dubick, B. A. Ryan, J. J. Summary and C. E. Wade, Dextran metabolism following infusion of 7.5% NaCl/6% Dextran-70 to euvolemic and hemorrhaged rabbits. *Drug Dev. Res.* **25**, 29–38 (1992).
34. J. G. Reinhold, C. A. J. Von Frijtag Drabbe, M. Newton and J. Thomas, Effects of dextran and polyvinylpyrrolidone administration on liver function in man. *Arch. Surg. AMA* **65**, 706–713 (1952).